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Availability of Elemental Selenium to Plants

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AVAILABILITY OF ELEMENTAL SELENIUM TO PLANTS

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TO PLANTS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of the thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Selenium has been found to be both essential for and toxic to animals and humans. Plants make up the main source of selenium for animals which are the food source for humans. Therefore, the selenium content in soils and plants is of interest.

The uptake and accumulation of selenium by plants is governed by many environmental factors such as plant species, soil pH, soil type, the chemical form of the selenium and the presence of other ions. The most important factors regulating selenium uptake by plants are the concentration and the form of selenium present in the soil.

Many authors have described the availability of selenium from different forms under various conditions. They found that the measurement of soluble selenium is the best relative index for the potential of selenium uptake by plants. Elemental selenium is very insoluble in water and is thought to be unavailable to crop plants.

Fly ash which is produced in coal-burning electric power-generating plants contains appreciable amounts of selenium which is mainly elemental selenium. It has been reported that plants grown on soils amended with fly ash absorbed significant amounts of selenium. For instance, white sweet clover grown on fly ash accumulated as much as 205 ppm selenium (Furr et al., 1978b). Generally, the uptake of selenium by plants has been correlated with the

amount of fly ash added.

In recent years, the emphasis of most studies on fly ash has been focused on the potential for using fly ash of known selenium content for increasing the selenium content of various crops to levels which would protect livestock from selenium-responsive diseases. Application of selenium salts to soil as a means of providing supplemental selenium to livestock is of limited use due to the high cost of the selenium salts.

It has been reported that only very small amounts of elemental selenium can be solubilized and absorbed (Watkinson and Davies, 1967). Generally selenium from this source would represent a very small amount of the total selenium in the plant. On the other hand, Cary and Allaway (1969) have demonstrated that elemental selenium can be transformed into more soluble forms by reactions that reduce elemental selenium, improving the availability of elemental selenium.

The objectives of this work are to try to examine the availability of elemental selenium, to find the processes involved in the solubilization of the elemental selenium, and to determine the effect of drying on the selenium content of plants.

LITERATURE REVIEW

Selenium is widely distributed in small amounts in the earth's crust, having an average abundance around 0.09 ppm (Lakin, 1972). The selenium contents of a wide range of rocks, minerals, lunar and volcanic materials, soils, plants, fossil fuels, and various waters have been determined.

Selenium occurs in the earth's crust mainly associated with sulfide minerals or as selenides of silver, copper, lead, mercury, and nickel or other metals. Some of the highest selenium contents of sulfides are associated with uranium ores from the sandstone-type deposits in the Western United States (Coleman and Delevaux, 1957). Goldschmidt (1954) has suggested that in high-temperature hydrothermal sulfides, the selenium content is higher than in low-temperature sulfides. Takimoto et al. (1958) concluded that the selenium content of sulfide materials is highest in those of earliest formation which may be related to the highest temperature of formation and not affected by the kind of sulfide mineral in which it occurs.

Because of the high levels of selenium in sedimentary rocks and the importance of such rocks as soil-forming materials, the processes which result in high selenium concentrations are of interest.

Lakin (1961) has suggested that selenium is concentrated in sedimentary rocks by the following processes: (1) precipitation by rain of selenium from volcanic emanations; (2) deposition of erosion products from volcanic sulfur, seleniferous tuffs, and sulfide deposits; and (3) precipitation of selenium from streams or groundwater carrying unusual quantities of selenium from older seleniferous sediments.

As indicated by Lakin and Davidson (1967), the selenium level in soils is related to the whole geochemical cycle of selenium and the solution chemistry of the element. The important factors include the selenium content of parent rocks, redox potentials, pH, solubility and the nature of the drainage waters (Allaway et al., 1967; Cary et al., 1967; Lakin, 1961; Allaway, 1968; Geering et al., 1968). Rosenfeld and Beath (1964) have pointed out that selenium in the soil may be derived from (1) rock formations or outcrops, (2) formations lying beneath the soil mantle, (3) decomposition of parent rocks by wind and water and subsequent transport by ground or surface water, (4) indicator plants, or (5) man-made enrichment of the soil with selenium in mining operations and as indicated by Lakin and Davidson (1967), the combustion of fossil fuels. The selenium content of most soils is between 0.1 and 0.2 ppm (Swaine, 1955).

Because of the small amounts of the element present

and the complexities of soil systems, selenium compounds in soils are difficult to separate and identify (Trelease and Beath, 1949; Allaway et al., 1967; Rosenfeld and Beath, 1964). The results for selenium from the analysis of compounds in soils suggest that there are differences in their solubility in water and in some other solvents. Selenium in the soils may be present as selenides, selenites, selenates, elemental selenium, and organic selenium compounds. Some of these forms are readily absorbed by all types of vegetation.

Soils that supply sufficient selenium to produce toxic plants are commonly referred to as toxic seleniferous soils. Nontoxic seleniferous soils contain insufficient available selenium for plants to absorb toxic levels.

Although selenium has not been found to be essential for plant growth, it is an essential element for animals (Underwood, 1971), and particularly grazing ruminants can show chronic symptoms of either toxicity or deficiency depending on the selenium concentration in their diet. For this reason the relationship between soil and plant selenium and the development of rapid availability tests for soil selenium are important and require elucidation before the supply of selenium via the pasture plants can be established and controlled.

The uptake of Se by plants is dependent upon the oxidation state in which the Se is present in the growing

medium. Ganje and Whitehead (1958) found a higher concentration of selenium in the plants when selenate was added to the soil than when selenite was added. The lower uptake of selenite from soil appears to be a consequence of the lower extractablility of selenite than of selenate (Cary and Gissel-Nielsen, 1973).

Grant (1965) and Watkinson and Davies (1967) found that the uptake from elemental Se was negligible and Cary, Wieczarek and Allaway (1967) found that one of the reasons for the relatively low availability of selenite was its reduction to elemental selenium or selenides. The speed of this reaction varies from one soil to the another but is apparently independent of the soil pH (Cary and Allaway, 1969).

Factors influencing the selenium content of plants have been reviewed by Johnson et al. (1967) including the species of plant, environmental factors, age, and phase of growth of the plant and nature of selenium compounds. One of the most important of these is the kind of plant. Rosenfeld and Beath (1964) have divided plants into three groups on the basis of their ability to accumulate selenium when grown on high selenium soils. Group 1 plants are referred to as selenium accumulator or indicator plants. They have the ability to accumulate selenium from the soils at very high levels, often over 1000 ppm. Some have thought that selenium indicator plants play an important part in the

interconversion of soil selenium forms. These plants absorb selenium from geological formations and convert it into soluble compounds which are readily available for absorption by all types of plant vegetation. However this role is no longer stressed (NRC, 1976). Plants in the second group are referred to as secondary selenium absorbers (Rosenfeld and Beath, 1964). They do not require selenium for their growth but when grown on soils of high selenium content they have the ability to accumulate selenium in concentrations of up to a few hundred parts per million. Plants in group three include many weeds and most crop plants, grains and grasses which do not normally contain more than 30 ppm of selenium, most of which is associated with plant protein. In general, soils of higher selenium content produce plants of high selenium content, but because of the importance of the form of selenium in soils in determining its absorption, the relationship is not strict.

The availability of different forms of selenium compounds to crop plants and indicator plants was studied by Moxon, Olson and Searight (1950). Selenates were found to be more available to plants than selenites.

Selenate has been identified as the main water-soluble form of selenium in soil that is associated with the production of vegetation containing toxic quantities of the element (Lakin, 1972). Many soils of the world contain high levels of selenium but low levels of water-soluble selenium

and consequently do not produce vegetation that has a toxic selenium level for animals.

A number of investigators have reported that there are organic selenium compounds in the soil. (Beath et al., 1935; Byers et al., 1938; Williams and Byers, 1936) suggesting that soluble organic selenium compounds present in the soils are due to decay of seleniferous plants. Byers et al. (1938) confirmed that seleniferous plant decay contributes considerable amounts of soluble organic and inorganic selenium compounds to the soil. Part of these soluble selenium compounds may be removed from the soil by leaching in areas of high or moderate rainfall, or part of the organic selenium compounds may be volatilized as indicated by noxious odors characteristic of growing seleniferous plants as well as of decaying plants with high selenium content. The remainder of the free selenium stays in the soil in available form.

Subsequent studies have confirmed that considerable amounts of water-soluble selenium present in the soils were in organic combination (Beath et al., 1937; Olson and Moxon, 1939). Olson and Moxon (1939) presented data showing that water-soluble and organic selenium content of soils can be used as a relative measure of availability of selenium to plants.

A number of investigators indicated that insoluble selenides, associated with sulfides, may occur in some soils

(Williams and Byers, 1936; Trelease and Beath, 1949; Allaway et al., 1967). The low solubility of metal selenides, especially copper selenide, may lead to their persistence in agricultural soils (Allaway et al., 1967). Although redox potentials indicate that selenides would be oxidized to selenite in most soils, the rate of oxidation is probably sufficiently slow to effectively stabilize this form of selenium under some soil conditions (Cary et al., 1967).

A large fraction of the selenium in acid soils may occur as stable complexes of selenites with hydrous iron oxides (Williams and Byers, 1936; Trelease and Beath, 1949; Swaine, 1955; Lakin, 1961; Allaway et al., 1967). Geering et al. (1968) showed that the thermodynamically stable selenium compound in acid to neutral soils may be a ferric selenite-ferric hydroxide adsorption complex. As the pH rises above 8, decomposition of the ferric hydroxide-selenite complex begins, and the equilibrium solubility of selenite increase rapidly. The rate of transformation of selenite to selenate proceeds very slowly.

Selenates have been reported to be present in water extracts of soil by several workers (Williams and Byers, 1936; Byers et al., 1938; Olson et al., 1942; Beath et al., 1946; Trelease and Beath, 1949). According to Lakin (1961), selenates are stable in an alkaline, oxidizing environment such as well-aerated, semiarid seleniferous soils. Selenates do not appear to be present in appreciable

quantities in acid and neutral soils. Marked increases in selenium uptake by plants have resulted from application of soluble selenates to soils (Grant, 1965; Hurd-Karrer, 1935; Bisbjerg and Gissel-Nielsen, 1969; Gissel-Nielsen and Bisbjerg, 1970).

Very little is known about the nature of organic forms of selenium in soils. According to Cary et al. (1967), organic forms of selenium are probably more soluble under alkaline than under acidic soil conditions.

Elemental Se is insoluble in water, but by hydrolysis or bacterial action small amounts may be converted to available form for crop plants (Beath et al., 1937). On the other hand Hurd-Karrer (1935) reported that elemental selenium was not absorbed by wheat from loam containing up to 200 ppm.

More recent work indicates that significant amounts of elemental selenium may be oxidized by microorganisms in neutral and alkaline soils (Geering et al., 1968). It may be either an important intermediate in the oxidation of the element to a soluble form (Olson, 1967) or a transitory constituent of neutral and acid soils during the reduction of selenites under acid conditions (Allaway et al., 1967).

The fate of elemental selenium in acid soils is unknown. Watkinson (1963) and Allaway et al. (1967) have suggested that when elemental selenium is added to acid and neutral soils. it may be oxidized to selenites, which in

turn react with hydrous oxides to form complexes of low solubility and availability to plants.

The principal factors affecting the availability of soil selenium to plants have been summarized as follows:

> (NRC 1971) In alkaline well-aerated soils Se tends to form selenates. The selenates in these soils are very available to plants and they may lead to toxic concentrations in plants. In acid soils a ferric iron selenite complex is formed that is only slightly available to plants. This is the reason acid soils rarely produce plants that contain toxic concentration of selenium. Elemental selenium appears to be stable in soils and except for microbial action, is not readily oxidized to forms that are easily taken up by plants. (Watkinson and Davies, 1967; Cary and Allaway, 1969).

Fly ash, which is produced by coal-burning electric power plants, was found to contain appreciable amounts of selenium (Furr et al., 1977). Gutenmann et al. (1976) found that fly ashes from 21 states contained 1.2 - 16.5 ppm of selenium. Most of the selenium may in fact be elemental selenium (Andren et al., 1975).

A very small proportion of all the fly ashes were recycled for beneficial use. In 1980, fifty-six million tons of coal fly ash were produced in the United States, but only 15% was utilized (NAS News Report, October, 1980). This material can be used in many ways (Brackett, 1970 and 1974; Capp and Spencer, 1970; Slonaker and Leonard, 1974) as a base material in road construction, in the manufacture of bricks, cement, ceramics and as an asphalt filler and a concrete additive. Although a small amount of fly ash is used as a road construction and concrete additive, most fly

ash is disposed of in landfills or settling ponds at costs of $0.25 - $2.00 per ton (Martens et al., 1970; Capp and Spencer, 1970).

High selenium content and plentiful supply have made fly ash attractive as a potential source of selenium for agricultural purposes in low-selenium areas such as the northeastern United States (Mbagwu, 1983).

The chemical form of selenium in fly ash is a matter of speculation. Andren et al. (1975) suggested that the environmental fate of selenium emitted by power plants, will depend on its chemical state. The expected forms of selenium in emissions from combustion processes are elemental selenium (Se^0), selenium dioxide (SeO_2), selenite ($SeO_3^=$) and selenate ($SeO_4^=$). It has been stated by Andren et al. (1975) that all of the selenium in the fly ash exists as elemental selenium possibly as a result of the reduction of tetravalent forms of selenium (SeO_2, H_2SeO_3) by sulfur dioxide generated during coal combustion. Subsequently, it is not immediately available for plant uptake.

However, agronomic consideration in using fly ash as a soil additive has been discussed (Capp and Spencer, 1970; Martens et al., 1970; Plank and Martens, 1973; Patterson et al., 1968; Plank et al., 1975). Several reports have shown that at least a portion of the selenium in fly ash is available for uptake by plants grown naturally or experimentally on fly ash or on soils amended with fly ash.

(Furr et al., 1975, 1976, 1977, 1978a,b; Gutenmann et al., 1976; Stoewsand et al., 1978). Selenium was consistently higher in all crops grown on fly ash amended soil and its concentration in the plants was approximately proportional to the rate of fly ash addition (Gutenmann et al., 1979).

Large areas in many parts of the world produced forages with selenium levels considered to be too low to meet the dietary needs of animals (Underwood, 1971). Fly ash therefore may be a useful soil amendment in agriculture.

Several workers reported that selenium occurring as selenide or in the elemental forms is very insoluble and is not readily absorbed by plants (Ganje and Whitehead, 1958; Hurd-Karrer, 1937; Moxon et al., 1950). It has been indicated that colloidal elemental selenium can be absorbed in very small amounts and solubilized by non-accumulators. (Watkinson et al., 1967; Peterson et al., 1966; Gissel-Nielsen and Bisbjerg, 1970; Butler and Peterson, 1967)

It has been reported that some elemental selenium can be converted to available form (Cary and Allaway, 1969). These authors pointed out that some of the elemental selenium which was added to soils was rapidly oxidized to selenite. After the occurrence of oxidation the remaining elemental selenium is quite inert and its oxidation proceeds very slowly.

Oxidation potentials for selenium as a function of pH indicate that colloidal elemental selenium would be

oxidized by the atmosphere to selenite (Se^{+4}) in alkaline to mildly acid conditions but further oxidation to selenate (Se^{+6}) would not occur (Lakin, 1961). The oxidation of selenium in soils is difficult to measure because selenite when formed is readily adsorbed onto hydrous iron oxide in the clay fraction. This form of selenium is unavailable to plants. (Watkinson, 1963)

In 1964, Shrift proposed that there may exist a natural biological selenium cycle just as cycles of carbon, nitrogen, and sulfur. The important point in this cycle is the reactivation of elemental selenium by microbial action. However, Allaway (1968) found that a part of the oxidation of elemental selenium appeared to proceed by a strictly non-biological pathway.

In an attempt to confirm the availability and pathway for solubilization of elemental selenium in soils, the objectives of this study are:

A) To examine the availability of elemental selenium to plants;

B) To determine the effect of drying on the selenium content of plants;

C) To examine the processes involved in solubilization of selenium solubilization in soils.

MATERIALS AND METHODS

Soils:

The first phase of this experiment was carried out in plastic trays (18 1/4" x 13 1/2" x 4"; L x W x H) each containing 18.2 kg of soil. The soil used in this experiment was a composite of local soil and was sandy clay loam texture (30% clay) with 6.4 pH.

Selenium Amendment:

A 2:1 soil-sand blend was thoroughly mixed with the elemental selenium preparation (see preparation in later section) in a cement mixer in 90.8 kg batches. The following concentrations were added in the soil: 0, 5, 10 ppm Se. Each batch was evenly divided among 5 trays. Therefore, two mixing batches were needed for each selenium level.

Soil Samples:

As the soils were transferred from mixer to plastic trays, samples were collected for analysis of the selenium content of the soil at the initial stage. These soil samples were air-dried and stored at room temperature. After harvesting, each tray was individually mixed using a Hobart mixer. Then soil samples (about 100 g) were collected for analysis of the selenium content at the final stage.

Plant Growth:

Wheat (Triticum aestivum, cult. Marshall) was chosen to be used in this study since it is easily grown under the conditions used. Three rows were planted in each tray and the seeds were separated by 1.5 inches. The seeds were covered with 1 inch of soil and deionized water was added to maintain moisture. The trays were stored inside a temperature controlled building until germination was complete. After one week, the trays were moved outside to a cement patio. The trays containing various treatments were randomly arranged in three rows of 10 trays each. They were watered daily with deionized water, to reach approximate field compacity (500-750 ml/day), and the weeds were removed. At 4 week intervals nitrogen was added to the trays in the form of urea and ammonium nitrate solutions. This solution was made by dissolving 3.24 g of urea and 4.29 g of ammonium nitrate into 18.9 l of deionized water. The nitrogen concentration of this solution was 0.42 g/l. In order to prevent the trays from flooding, as there were no drainage openings, a plastic shelter was placed over the plants when it rained. The shelter was A-shaped and open on the ends to allow complete air movement.

Plant Harvesting:

The plants were grown over a twelve week period. The first sampling was made when approximately 50% of the plants were in the boot stage (42 days). Each tray was

sampled as follows:

1) The two outside rows were clipped 1 cm above the soil surface.

2) Leaves touching the soils were not sampled, only standing leaves were taken.

3) Four weighed paper bags were used for each tray. The first plant was placed in the first sack, the second plant in the second sack, etc. The fifth plant was placed in the first sack again, etc., until all the plants in the two outside rows were removed. Each sack was weighed immediately after harvesting.

4) Prior to analysis for selenium, any residual soil was removed from the plants by the following washing procedure:

a) The wheat plants were removed from the sacks and grasped by the cut ends and immersed in a pan of water containing dilute Alconox.

b) Subsequently, they were dipped at least twice in a pan containing deionized water.

c) They were then blotted dry on paper towels.

5) Two sets of samples to be dried, were placed back into the original sack which was closed loosely. Then they were dried for 24 hours at 70^0C in a forced air oven.

6) Two sets of samples to be analyzed directly, were placed wet into flasks and predigested with nitric acid. They were made to a total volume of 50 ml. Comparison of

the selenium content between sample sets in 5 and 6 should provide information on selenium losses due to drying.

The wheat in the center row of each tray was harvested when the plants had reached maturity stage and the grain and straw were separated. The straw was dried at 70^0C in a forced air oven for 24 hours.

Method of selenium analysis:

Soil and plant samples were first predigested in concentrated nitric acid and made to volume. An aliquot was digested in a 4:1 nitric/perchloric acid mixture. Digestion was continued to the white fume stage and then the samples were reduced by adding concentrated HCl and heating. Total selenium was determined using 2,3-diaminonaphthalene as a complexing agent to form a piazselenol which was extracted in cyclohexane (Koh and Benson, 1983) and the selenium content of the extraction was measured fluorometrically using a Perkin/Elmer LS-2B Filter Fluorimeter at excitation wavelength 350 nm and emission wavelength 550 nm.

Water soluble selenium in soils was determined by the following procedure: Soils were extracted with $CaCl_2$ (5 g soil: 35 ml, 0.1 M $CaCl_2$) for 2 hours on a shaker. The suspensions were centrifuged, and an aliquot was filtered through a 0.45 micron filter and was digested and treated as other samples for selenium analysis.

Preparation of Elemental Selenium:

Colloidal Selenium Preparation: Colloidal elemental

selenium was prepared by dissolving 21.90 g sodium selenite within a slurry of 500 g of silicic acid in 1000 ml of deionized water. Hydroxylamine was added slowly until no further formation of elemental selenium was observed. The preparation was washed with sufficient 0.1 N HCl to remove all traces of water soluble selenium.

Determination of Water Soluble Selenium in Selenium Preparation: Water soluble selenium in the selenium preparation was determined by a procedure based on the study of Mason (1981). A 0.5000 g sample of selenium preparation was weighed into a 250 ml Teflon beaker. Five ml of water and 9 ml of HF were added to the sample. The mixture was stirred on a magnetic stirrer for 30 minutes and then allowed to stand for at 1 hour. The solution was filtered with fine filter paper into a Teflon beaker and washed with 1% HF. The volume was made up to 50 ml. Four grams of H_3BO_3 was added to the filtrate. After dissolution of the H_3BO_3 the selenium content of the filtrate was determined. Elemental selenium would be retained on the filter.

Total Selenium in the Selenium Preparation: A 0.5000 g sample of the preparation was predigested with concentrated nitric acid and diluted with deionized water to 1000 ml. A 1.0 ml aliquot was diluted to 10 ml in a volumetric flask. Selenium was determined as in the other samples.

Determination of Mechanism for Solubilization of Selenium in Soil:

In order to further examine the conversion of elemental selenium to available forms, a mixture of soil and selenium preparation was subjected to various treatments and increases in soluble selenium were measured. The original soil was autoclaved in 500 g batches at 121°C and 15 psi for 45 minutes to completely destroy the microorganisms in the soil. This soil sample was stored at room temperature. After 24 hours, the autoclave procedure was repeated again. The autoclaved soil was mixed with 0.186 g of colloidal selenium preparation (1.0 g of selenium preparation has 13450 ug of elemental selenium). The soil-selenium mixture was separated into two parts. One of the parts was again divided and the subsamples were placed in previously autoclaved glass containers. Both of the subsamples were flushed with nitrogen and one was stored at room temperature (20°C), the other in a freezer (-10°C). The second part of the soil-selenium mixture was not flushed with nitrogen, but was divided and stored in the two different temperature environments in autoclaved containers. The nonautoclaved soils were subjected to the same procedures.

Soluble selenium content was measured at 4 week intervals. To determine the initial mixing effect, the soils were analyzed immediately after mixing with the

selenium preparation. Only two batches of soils were analyzed for inital content of soluble selenium at zero time. One was autoclaved and the other was not. Neither were flushed with nitrogen or stored since this procedure was performed at 16 weeks on samples that were not used in the treatments.

Statistical Analysis:

The data were analyzed by a nested analysis of variance in all cases except in the study concerning the mechanisms of selenium solubilization. Paired-t test was used to compare the inital and final Se content of soil.

RESULTS AND DISCUSSION

Growth of Wheat in Soil Amended With Elemental Selenium

In general, the wheat plants grew well in the trays. The weight of straw and grain at final harvest are shown in Table 1. On the basis of total dry weight produced per tray, there were no significant differences between control and treatment groups. Under the conditions of this study, no toxicity to the plants was evident even at 10 ppm added selenium.

The amount of elemental selenium added to the soil and the results of selenium analysis of the entire plant are given in Table 2. All the plants were collected at the green stage of growth where 50% were starting to head. Half of the plants were analyzed immediately and half were dried and then analyzed. The selenium content was converted to a wet weight basis. The moisture contents of the freshly harvested plants varied from 71.9 to 77.6 percent.

It can be seen that the selenium content of dried plants and fresh plants were not significantly different ($p \leq 0.05$). For instance, the mean of selenium contents in the immature wheat grown on soils with 10 ppm of added selenium were 3.72 ppm and 3.77 ppm respectively. At the 5 ppm added elemental selenium level, there was a numerical difference between mean selenium content of dried and wet samples that was not significant ($p \leq 0.05$) probably because of the large standard deviation for one set. It should be noted that

(Table 1) Weight of straw and grain for experimentally grown wheat[a]

	Straw[b]	Grain[b]
Control	12.36 ± 1.84	6.23 ± 1.23
5 ppm	11.47 ± 1.88	5.63 ± 0.76
10 ppm	11.90 ± 2.44	6.45 ± 1.38

[a] Comparing treatment group with the control by paired-t test showed no significant difference in weights ($p \leq 0.05$).

[b] Values are means from 10 trays (in grams) ± SD.

(Table 2) Se content in the wheat samples at 42 days (wet basis)

	Se content (ppm)[a]	
	Before drying	After drying
Control	0.068 ± 0.008	0.068 ± 0.007
5 ppm	1.54 ± 0.43	1.69 ± 0.15
10 ppm	3.77 ± 0.36	3.72 ± 0.40

[a] Dried and fresh plants were not significantly different at $p \leq 0.05$ level.

even if the difference had been significant, the selenium content was higher for the samples exposed to drying. It can be concluded that drying fresh cereal vegetation at 70°C does not result in the loss of selenium.

This data is in agreement with the results of Beath et al. (1937). They discovered that the drying of certain primary indicator plants was accompanied by loss of a large amount of selenium, indicating that a volatile selenium compound was present in these plants. However, they found no such loss on drying grass or cereal crops.

The data in Table 3 show the accumulation of selenium in the two portions of wheat, grain and straw. Those plant samples were collected at the maturity stage and analyzed. Amounts of selenium present are expressed based on oven dried weights. The data show that there is a linear relationship between soil selenium and plant selenium. For example the selenium content in the grain increased from 12.98 to 27.03 ppm as the selenium content in the soil increased from 5 to 10 ppm. The selenium content of the straw was lower, increasing from 9.63 to 23.03 ppm, respectively.

Most of the selenium in non-accumulating species has been found associated with the protein (Franke and Painter, 1936; Horn et al., 1936; Butler and Peterson, 1967). Since protein content of grain is usually higher than in straw, the data presented here was expected. Others

(Table 3) Selenium content of harvested wheat (dry basis)

	Se content (ppm)	
	Grain	Straw
Control	0.38 ± 0.05	0.30 ± 0.03
5 ppm	12.98 ± 1.07	9.63 ± 1.69
10 ppm	27.03 ± 2.82	23.03 ± 7.00

have found that the selenium content at maturity was less in the straw than in the grain regardless of the amount of selenium present in the soil (Johnson and Whitehead, 1951; Rosenfeld and Beath, 1964).

It was surprising to find such high selenium levels in plants grown on the soils amended with just elemental selenium. The uptake of selenium by plants is governed by the presence and availability of selenium in the soil. It has been suggested by Gissel-Nielsen and Bisbjerg (1970) that the form and solubility of the element is the most important factor in determining plant uptake. As discussed in the literature review, the water soluble selenium seems to best represent the amount available to the plants.

In order to better understand the reasons for the high uptake of selenium from soils amended with elemental selenium, the amount of water soluble and total selenium in the soils was determined at the beginning and the end of the wheat growth experiment. The results are given in Table 4. The initial and final concentration of total selenium and soluble selenium at each treatment level were compared by paired-t test. The total selenium for each level tended to decrease with time increased. For example, for the 10 ppm level, the amount of total selenium at the initial stage was 11.43 ppm. At the end of this experiment, the amount of total selenium significantly decreased ($p \leq 0.01$) to 8.35

(Table 4) The total Se and water soluble Se in the soil[a]

Treatment	Total Se (ppm) Initial	Total Se (ppm) Final
Control	0.20 ± 0.06	0.23 ± 0.03
5 ppm	5.23 ± 1.42	4.15 ± 0.27*
10 ppm	11.43 ± 2.22	8.35 ± 0.26**

Treatment	Water soluble Se (ppm) Initial	Water soluble Se (ppm) Final
Control	0.0066 ± 0.002	0.014 ± 0.003**
5 ppm	0.017 ± 0.002	0.080 ± 0.007**
10 ppm	0.023 ± 0.002	0.17 ± 0.023**

[a] The final levels of total selenium and water soluble selenium in each pot were compared to initial values by paired-t test. *$p \leq 0.05$; **$p \leq 0.01$.

ppm. The same trend was observed from the soil amended with 5 ppm selenium ($p \leq 0.05$). The content of total selenium for the control group did not significantly vary between the two stages.

Attention should be drawn to the concentration of water soluble selenium in these soils (Table 4). The data show that amending the soil with the elemental selenium preparation did increase the amount of soluble selenium (see the initial values). The 0.023 ppm soluble. selenium obtained at the 10 ppm treatment level, although three times the level in the control, is still well below the level of 0.1 ppm that would be expected to increase plant content (Palmer, 1988). At all these levels of added elemental selenium, the amount of water soluble selenium at the final stage was significantly higher ($p \leq 0.01$) than at the beginning of the experiment. Table 4 reveals that at the highest level of supplementation, the soluble selenium increased to 0.17 ppm. This is normally considered sufficient to produce vegetation which might contain toxic levels of selenium.

From the statistical analysis of the data, (Tables 4a and 4b) it is evident that the soil preparation was done adequately, since no significant difference ($p \leq 0.05$) was found between the total selenium levels in the different mix batches. The same is true for soluble selenium. Although a significant tray effect was detected in two instances

(Table 4a) Analysis of variance and means for total selenium in soil.

Source	Initial Se Concentration df	MS	F	Final Se Concentration df	MS	F
Level	2	632.23	78.56**	2	329.52	9836**
Batch (Lev)	3	8.05	2.08	3	0.0335	0.56
Tray (Lev x Bat)	24	3.88	6.72**	24	0.059	0.98
Residual	30	0.58		30	0.06	

Inital mean selenium concentration (ppm).

Level	Overall	Batch 1	Batch 2
Control	0.20	0.21	0.19
5 ppm	5.23	4.19	6.27
10 ppm	11.42	11.06	11.79

Final mean selenium concentration (ppm).

Level	Overall	Batch 1	Batch 2
Control	0.24	0.225	0.245
5 ppm	4.15	4.09	4.21
10 ppm	8.35	8.32	8.39

* Significant at $p \leq 0.05$.
** Significant at $p \leq 0.01$.

(Table 4b) Analysis of variance and means for soluble selenium in soil.

Source	Initial Se Concentration df	MS	F	Final Se Concentration df	MS	F
Level	2	0.001306	480**	2	0.119	476**
Batch (Lev)	3	0.00000272	0.35	3	0.000250	0.51
Tray (Lev x Bat)	24	0.00000766	0.41	24	0.000488	5.02**
Residual	29	0.0000187		30	0.00009713	

Inital mean selenium concentration (ppm).

Level	Overall	Batch 1	Batch 2
Control	0.0066	0.0062	0.0069
5 ppm	0.0169	0.0165	0.0172
10 ppm	0.0225	0.0221	0.0229

Final mean selenium concentration (ppm).

Level	Overall	Batch 1	Batch 2
Control	0.0132	0.0133	0.0131
5 ppm	0.0764	0.0752	0.0776
10 ppm	0.167	0.173	0.161

* Significant at $p \leq 0.05$.
** Significant at $p \leq 0.01$.

(initial values for total selenium and final value for soluble selenium), the difference was of no practical importance.

The increase in soluble selenium in the soil preparation, with subsequent plant uptake, could be due to a true solubility of the elemental selenium or it might represent the freeing of adsorbed selenite in the selenium-silicic acid preparation. The data in Table 4 indicate that a small amount of soluble selenium was contributed by the preparation (0.02 ppm) in soils amended to 10 ppm. This was about 3 times the detected level in the 0 level treatment but would still be inadequate to explain the elevated plant selenium content. However, it appeared conceivable that any adsorbed selenite might not have been totally removed by our method of washing the selenium preparation.

A further attempt was made to characterize the form of selenium in the Se-silicic acid preparation using the method of Mason (1981). Samples of the preparation were dissolved in HF to destroy the silicic acid matrix and dissolve all non-elemental selenium. The elemental selenium could then be removed by filtration and available selenium determined on the filtrate.

The results are seen in column 3 of Table 5. From a 0.5 g sample, 2.69 $\pm$ 0.24 ug of selenium was recovered as soluble selenium. Since the total selenium content in the preparation was 6725 ug/0.5 g, the percent of soluble

(Table 5) ug Soluble Se from selenium-silicic acid preparation

Rep.	Sample wt.	Se in original sample	Se in spiked sample[a]	Net recovery	% recovery
1	0.5000 g	2.39[b]	4.71[b]	2.32	116.0
2	0.5000 g	2.81	4.89	2.08	104.0
3	0.5000 g	2.88	4.82	1.94	97.0
Overall		2.69 ± 0.24	4.80 ± 0.15		105.7 ± 10.1

[a] 2 ug Se added as selenite

[b] All values are averages of duplicate determinations

selenium was 2.69/6725 x 100 = (0.04%). This would be the equivalent of adding 0.004 ppm to the trays containing 10 ppm added selenium.

To more directly determine that adsorbed selenite could be detected by the methods used, other samples of the preparation were spiked with 2 ug selenium as selenite prior to treatment with HF. Data in column 4,5 and 6 of Table 5 show that 105.7% ± 10.1 of the added selenite could be recovered. These data indicate the method used here is adequate to detect non-elemental selenium present in the preparation.

Conversion of Elemental Selenium to Soluble Forms in Soil

Oxidation of the applied elemental selenium is assumed to be necessary for its utilization and this may occur in soil. This conversion has been shown by Geering et al. (1968). They added 6 ppm colloidal selenium to soils with 3 different pH levels (5.0, 5.9, 6.7). The amount of selenium oxidized increased with the pH. Part of the oxidation seemed to proceed by nonbiological pathways. Other evidence that elemental selenium can be converted to available forms has been supplied by Cary and Allaway (1969).

In an attempt to determine how the elemental selenium was converted to soluble forms in this study, the selenium-silicic acid preparation was added to a 500g batch of soil. The batch was divided and the parts were subjected

to different treatments including autoclaving, to determine whether microbial action was involved; flushing with nitrogen, to determine if just spontaneous oxidation was involved; and storage at two different temperatures. Samples of soil from the various treatments were taken at four weeks, eight weeks, twelve weeks and sixteen weeks and analyzed for soluble selenium as described previously. It should be noted that in all cases, the autoclaving was carried out before mixing the soil and selenium. The initial pH of the soil used was 6.4. The results are shown in Table 6.

It can be seen that the lowest amount of soluble selenium was obtained in nonautoclaved groups which were stored at -10° C. These groups represent the negative control and contained average soluble selenium levels of 1.475 to 1.725 ppb. Other treatments tended to result in increases of soluble selenium with the highest level obtained in the treatment which was not autoclaved, not flushed with nitrogen and was stored at room temperature. The production of the highest amount of soluble selenium in this group is indicative of the participation of microbial action in the solubilization process.

It appears that there was an increase in soluble selenium in all treatment groups at the four-week sample date as compared to the negative controls. The question arose as to whether or not this increase was instantaneous

(Table 6) Water soluble Se in the soil amended with 5 ppm Se from the Se-silicic acid preparation after several treatments[a]

	Time				
	0 weeks	4 weeks	8 weeks	12 weeks	16 weeks
			(ppm x 10^{-2})[b]		
Flushed with N_2					
Autoclaved					
20°C		3.7 ± 0.4	4.6 ± 0.6	4.5 ± 0.7	4.0 ± 0.1
-10°C		3.9 ± 0.2	3.6 ± 0.4	3.9 ± 0.2	2.8 ± 0.1
Nonautoclaved					
20°C		4.1 ± 0.3	3.7 ± 0.2	4.5 ± 0.3	4.5 ± 0.1
-10°C		2.3 ± 0.7	1.1 ± 0.2	1.8 ± 0.5	1.7 ± 0.2
Not flushed with N_2					
Autoclaved					
20°C	0.9 ± 0.1	5.3 ± 0.2	4.8 ± 0.2	5.3 ± 0.4	4.9 ± 0.3
-10°C		4.5 ± 0.6	3.6 ± 0.3	3.8 ± 0.4	2.5 ± 0.1
Nonautoclaved					
20°C	0.7 ± 0.2	2.3 ± 0.3	4.4 ± 0.3	6.0 ± 0.5	7.6 ± 0.9
-10°C		1.8 + 0.4	1.4 + 0.4	1.6 + 0.4	1.1 + 0.4

[a] Values are mean ± SD
[b] The actual value is obtained by multiplying the tabular value by 10^{-2}

or time dependent. Since zero time samples were not initially taken, it was not possible to determine this from the original experiment. Consequently, two other batches of selenium-soil mixtures were prepared. One was autoclaved and both were sampled immediately (zero time) and soluble selenium content was determined. It can be seen from the data in column one in Table 6 that soluble selenium was low initially (0.9 or 0.7 ppb). Apparently, a major portion of the increase in soluble selenium observed in the other treatment groups occurred in the first four weeks. Storage at -10^{o}C prevented most of the increase although soluble selenium even increased slightly in this treatment group in the first four weeks.

The data presented in Table 6 were analyzed for as a complete factorial analysis of variance and the analysis is shown in Table 7. Although there appear to be significant main effects, in most instances they can not really be discussed because of the significant interactions. In this study most of the treatments (flushing with N_2 (N), autoclaving (A), storage at different temperatures (T)) had similar effects over time (t) on the amount of soluble selenium. Therefore, one treatment was often adequate to produce maximum response and superimposing one or more treatments produced no further response and therefore a significant interaction was obtained. It appears a more useful presentation of the data involves breaking it down

(Table 7) Statistical analysis of experiment for conversion of elemental to soluble forms in soil

Source	df	ms ($x10^{-3}$)[a]	F
Nitrogen (N)	1	0.44293	31.28**
Autoclave (A)	1	3.01064	212.6**
N x A	1	0.01909	1.35
Temperature (T)	1	13.1281	27.1**
N x T	1	0.68273	48.21**
A x T	1	3.02977	214.0**
N x A x T	1	0.02064	1.46
Error(a)	8	0.01416	
Time (t)	3	0.15342	5.86**
N x t	3	0.00948	3.62*
A x t	3	0.60079	22.95**
N x A x t	3	0.39898	15.24**
T x t	3	0.79959	30.55**
N x T x t	3	0.26375	10.08**
A x T x t	3	0.14985	5.72**
N x A x T x t	3	0.19489	7.45**
Error(b)	24	0.02618	

[a] To obtain the mean square value the tabular value should be multiplied by 10^{-3}.

*$p \leq 0.05$;

**$p \leq 0.01$.

into smaller segments.

In Figures 1 - 6 the amounts of water soluble selenium in the soil extracts from the various treatments were plotted against the storage time. In some instances, the same data are presented on more than one Figure for the purpose of making easier comparisons. For instance, Fig.1b, 3b, and 6b all contain the data for soluble selenium in soil which was not autoclaved, not flushed with nitrogen but was stored at room temperature.

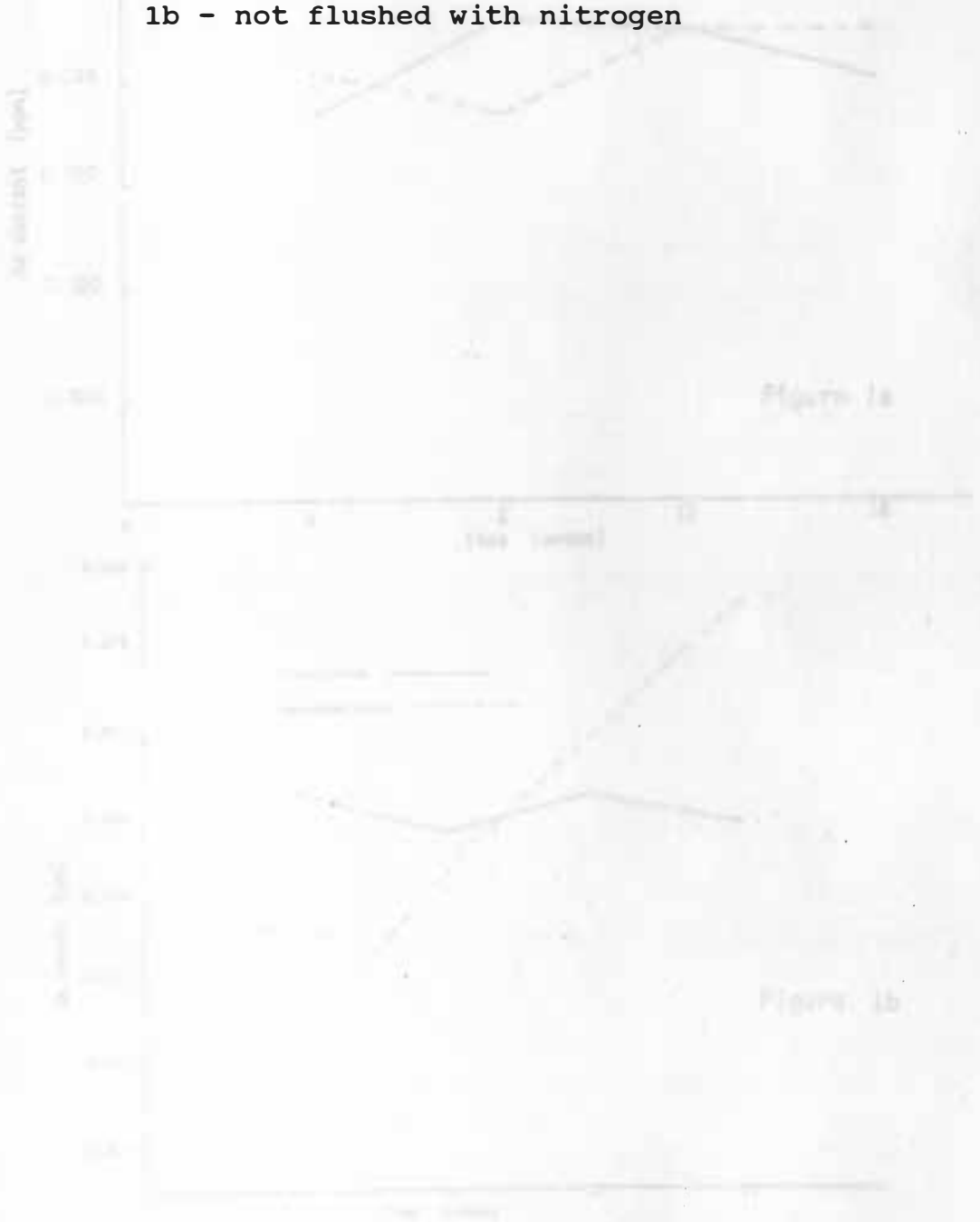
Figures 1 and 2 show the effect of autoclaving on the production of soluble selenium. Figure 1 shows data for soils stored at 20^{o}C and Figure 2 shows data for soils stored at -10^{o}C. Part a of each Figure shows data from samples flushed with nitrogen and Part b is for samples not flushed with nitrogen. There is fairly good consistency in the data. In Figures 1b, 2a, 2b, the soluble selenium in treatment groups which have been autoclaved is higher in all cases. The single exception to this observation is shown in Figure 1a. In the latter instance, the autoclaved and nonautoclaved groups were essentially the same in terms of soluble selenium level. At present, there is no explanation for this one exception. If this one observation is considered unusual, then it can be stated that autoclaving seems to produce a condition that causes an increase in soluble selenium. Again, it should be noted that the elemental selenium was not added to the soil until after the

Figure 1.

Effect of autoclaving on water soluble selenium in soil mixtures stored at 20°C.

1a - flushed with nitrogen

1b - not flushed with nitrogen



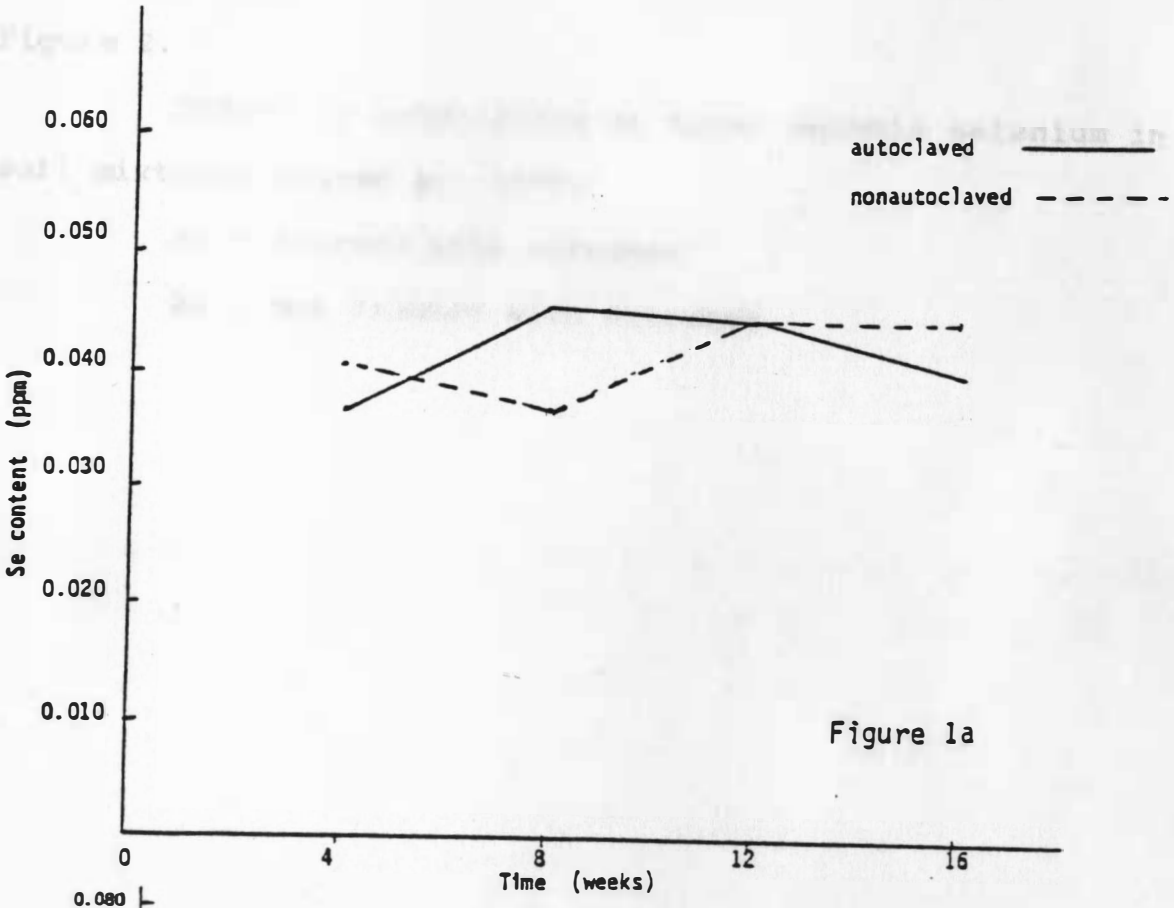


Figure 1a

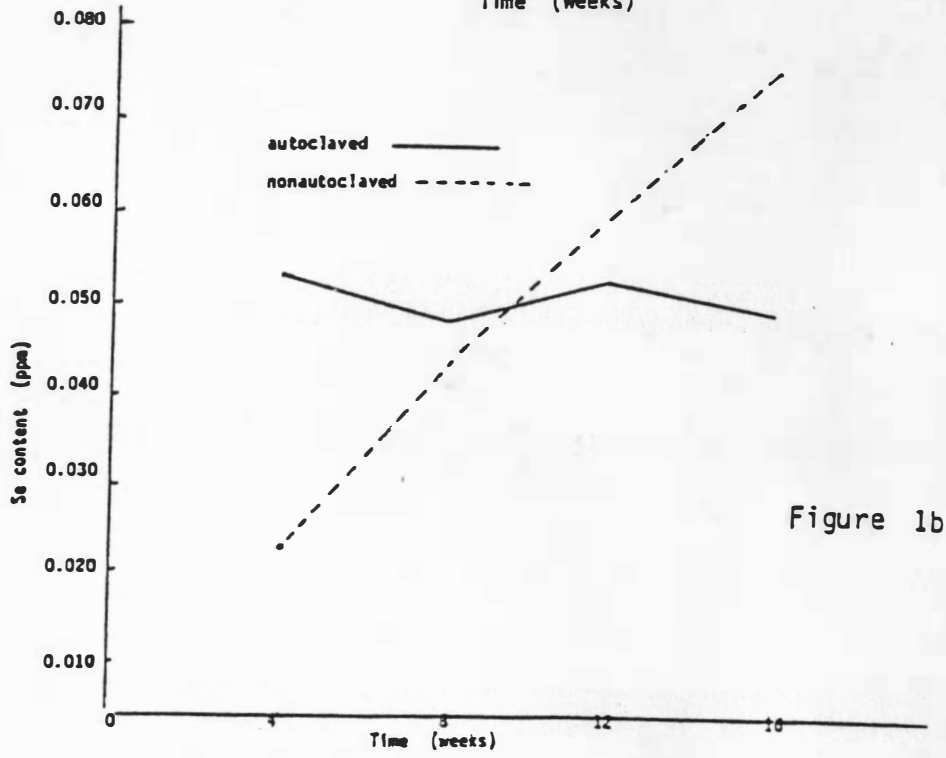


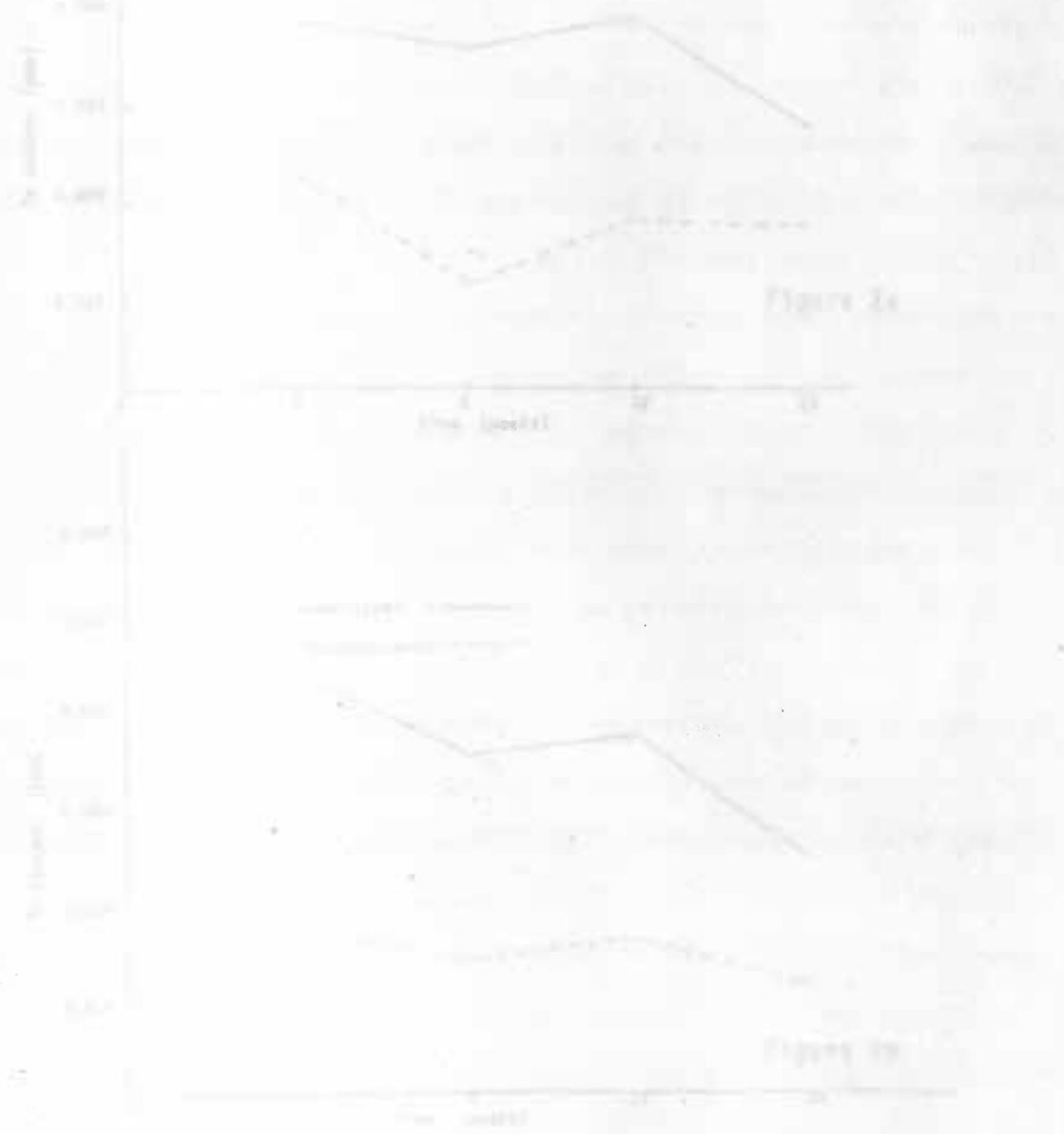
Figure 1b

Figure 2.

Effect of autoclaving on water soluble selenium in soil mixtures stored at -10°C.

2a - flushed with nitrogen

2b - not flushed with nitrogen



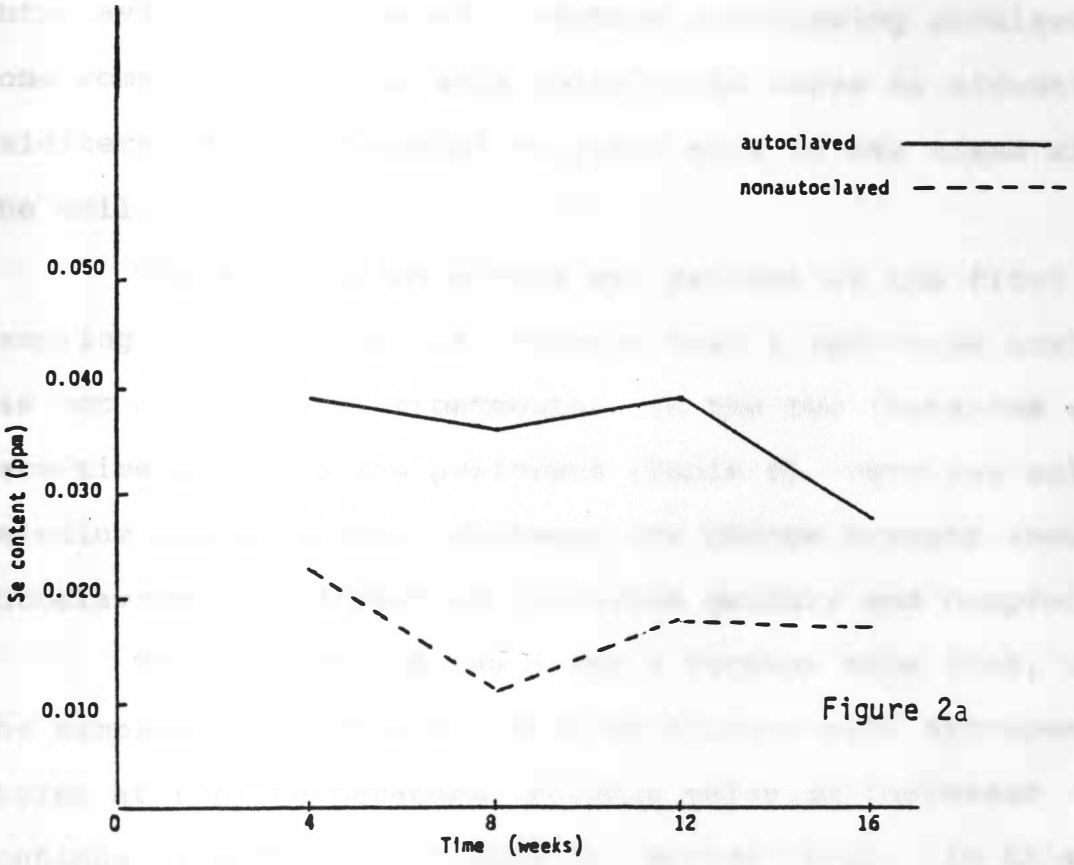


Figure 2a

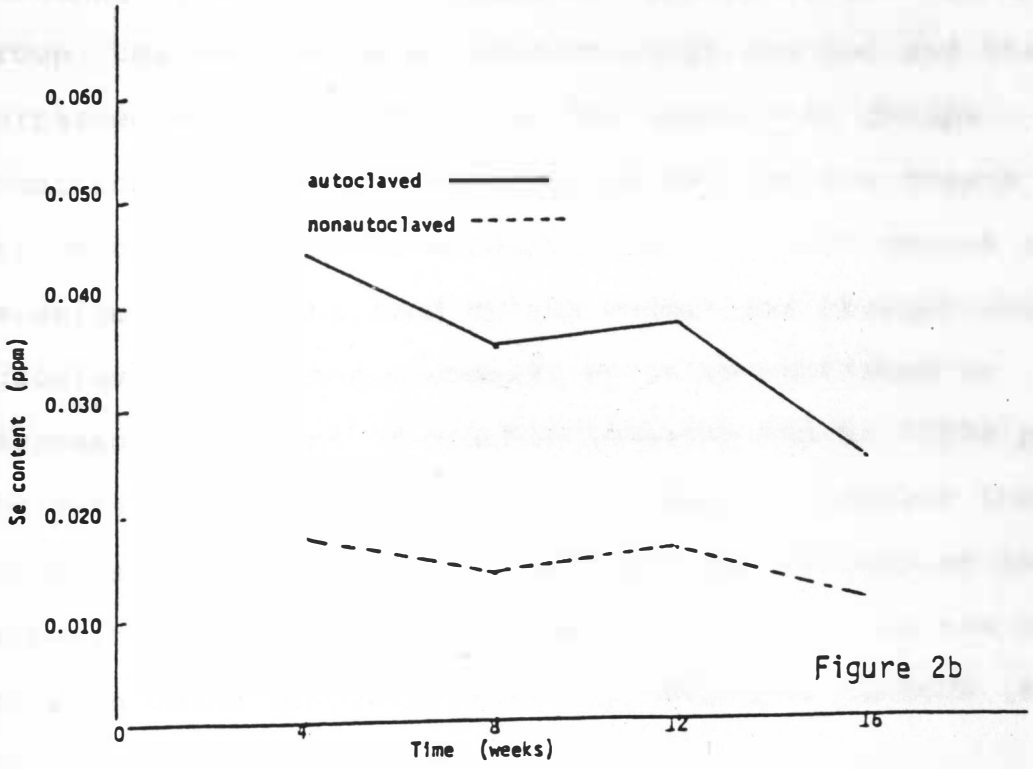


Figure 2b

autoclaving was completed. Perhaps autoclaving oxidized some components in the soil which could serve as effective oxidizers of the elemental selenium when it was mixed with the soil.

The autoclaving effect was present at the first sampling date. It was unfortunate that a zero-time analysis was not done for all treatments. In the two instances when zero-time analysis was performed (Table 6), very low soluble selenium was observed. Whatever the change brought about by autoclaving, its effect is exercised quickly and completely.

The data in Figures 1 and 2 further show that, when the samples were not autoclaved or flushed with nitrogen and stored at room temperature, soluble selenium increased continually with time (Figure 1b, dotted line). In this group, the soluble selenium eventually reached and then surpassed the levels found in the autoclaved groups. All condition in this particular group favored the growth of microorganisms. It is evident that a certain amount of selenium was solubilized by the conditions brought about by autoclaving and then microbial activity continued to increase the amount of soluble selenium (up to 0.076 ppm). The data as displayed in Figures 1 and 2, indicate that there is little effect of flushing with nitrogen or storage temperature on soluble selenium levels except in the one group in which microbial activity would be maximum (Figure 1b).

In Figures 3 and 4, the data are assembled to look at the main effect of flushing with nitrogen, thereby excluding oxygen. It should be understood that the methods employed did not completely remove the oxygen. Nevertheless, flushing with nitrogen generally kept soluble selenium at a level no higher than that produced by the autoclaving treatment. Again, it is evident that in the group in which the microorganisms' growth could be maximum since the soil was not autoclaved and was stored at ambient temperature (Figures 3a and 3b), the lack of nitrogen flushing allowed an increase in soluble selenium. This was probably due to microbial activity.

The data in Figures 5 and 6 are grouped so as to look at temperature as a main effect. Storage at -10°C resulted in the lowest content of soluble selenium in all cases. Even in the group not autoclaved and not flushed with nitrogen, storage at -10°C was sufficient to keep the soluble selenium level low.

In summary, it appears that elemental selenium, as prepared in this study, is more available to plants than previously thought. Its availability can be measured by plant uptake or an increase in soluble selenium in the soil. The solubilization of elemental selenium can be partially explained by a chemical reaction between the soil and selenium. Although the nature of the reaction is still unclear, it apparently is accelerated by treatments such as

Figure 3.

Effect of flushing with nitrogen on water soluble selenium in soil mixtures stored at 20°C.

3a - autoclaved

3b - nonautoclaved

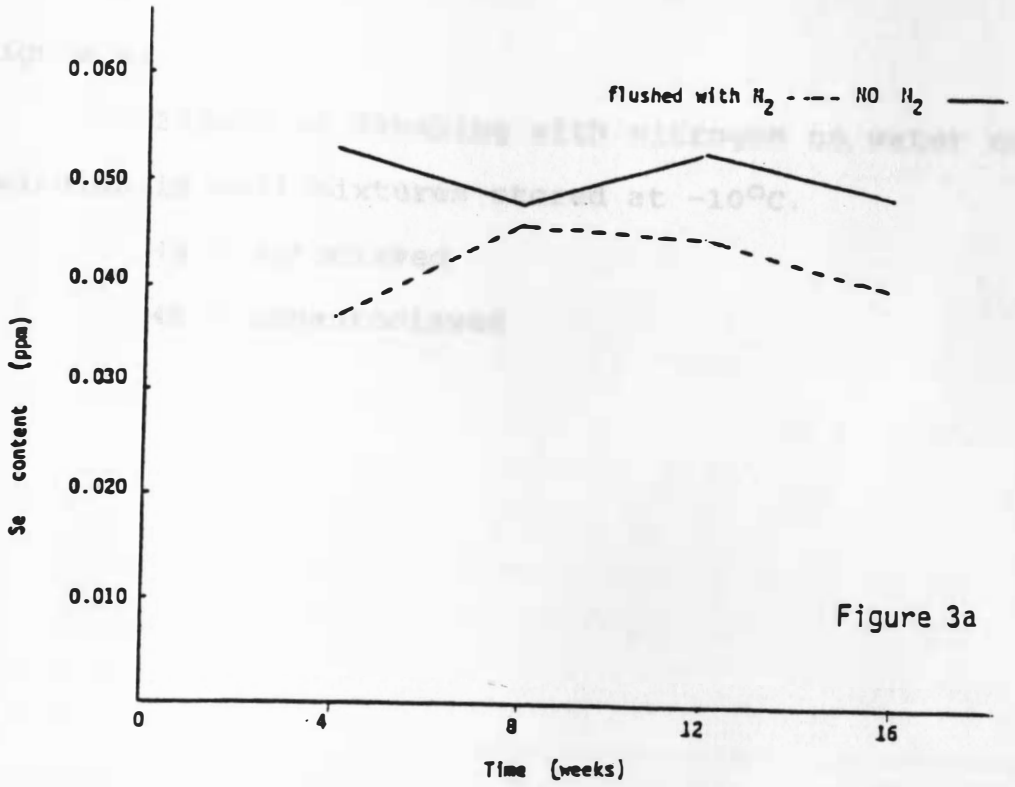


Figure 3a

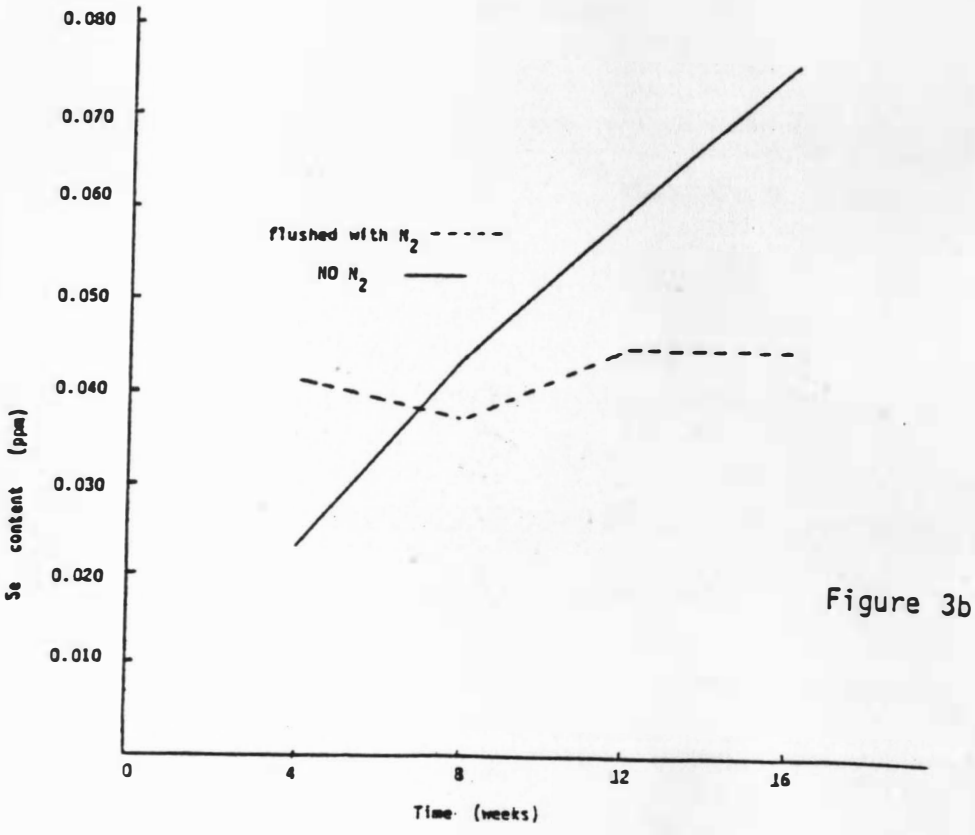


Figure 3b

Figure 4.

Effect of flushing with nitrogen on water soluble selenium in soil mixtures stored at -10°C.

4a - autoclaved

4b - nonautoclaved

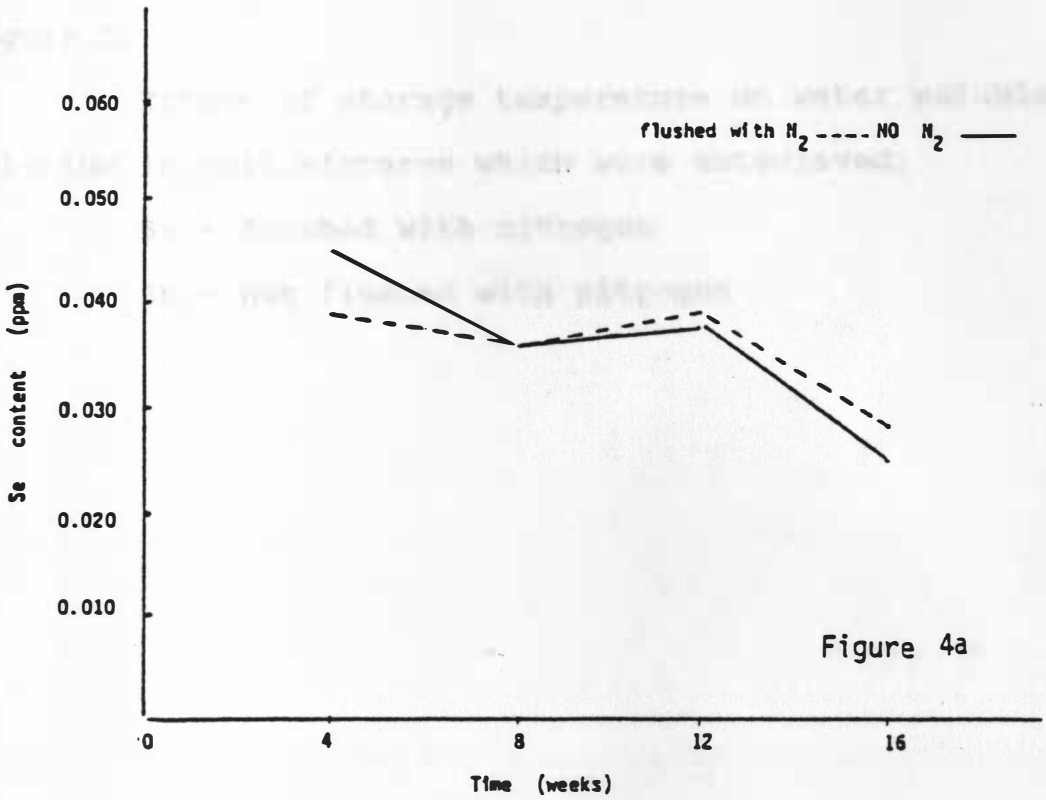


Figure 4a

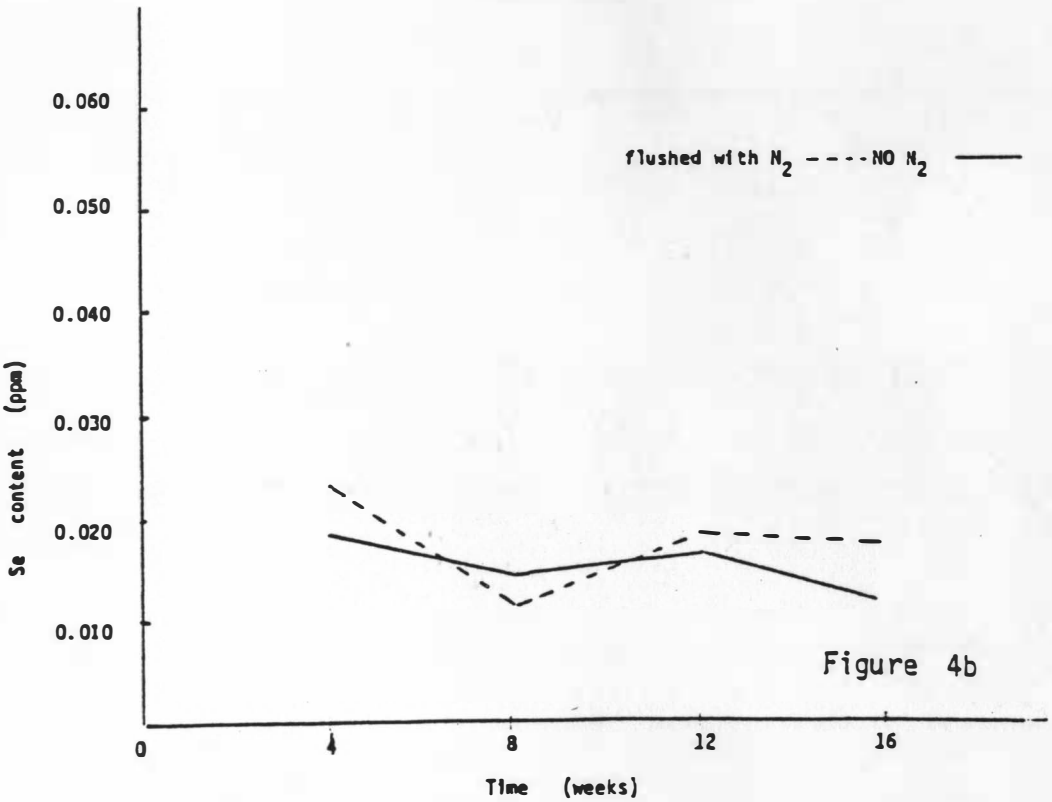


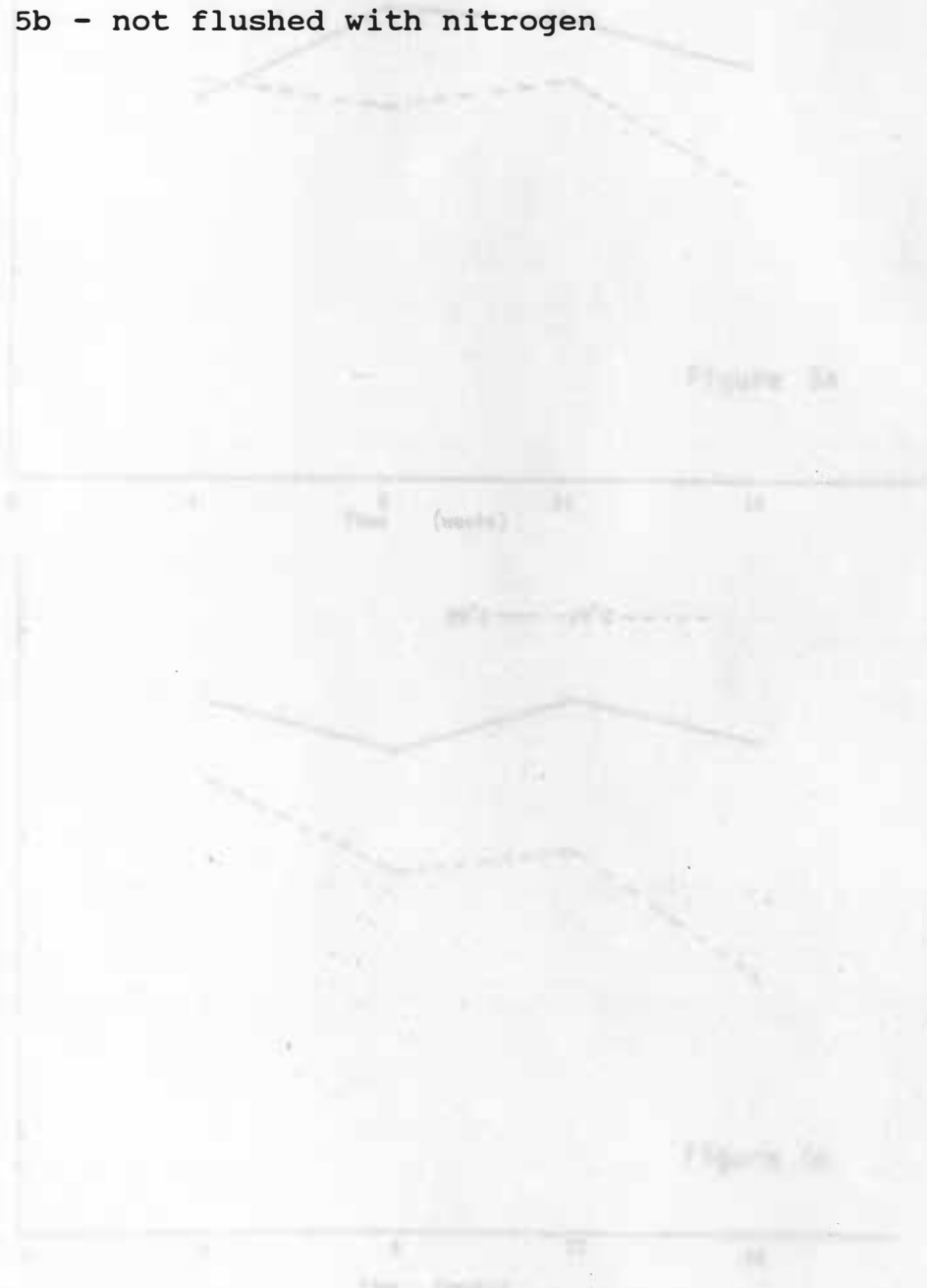
Figure 4b

Figure 5.

Effect of storage temperature on water soluble selenium in soil mixtures which were autoclaved.

5a - flushed with nitrogen

5b - not flushed with nitrogen



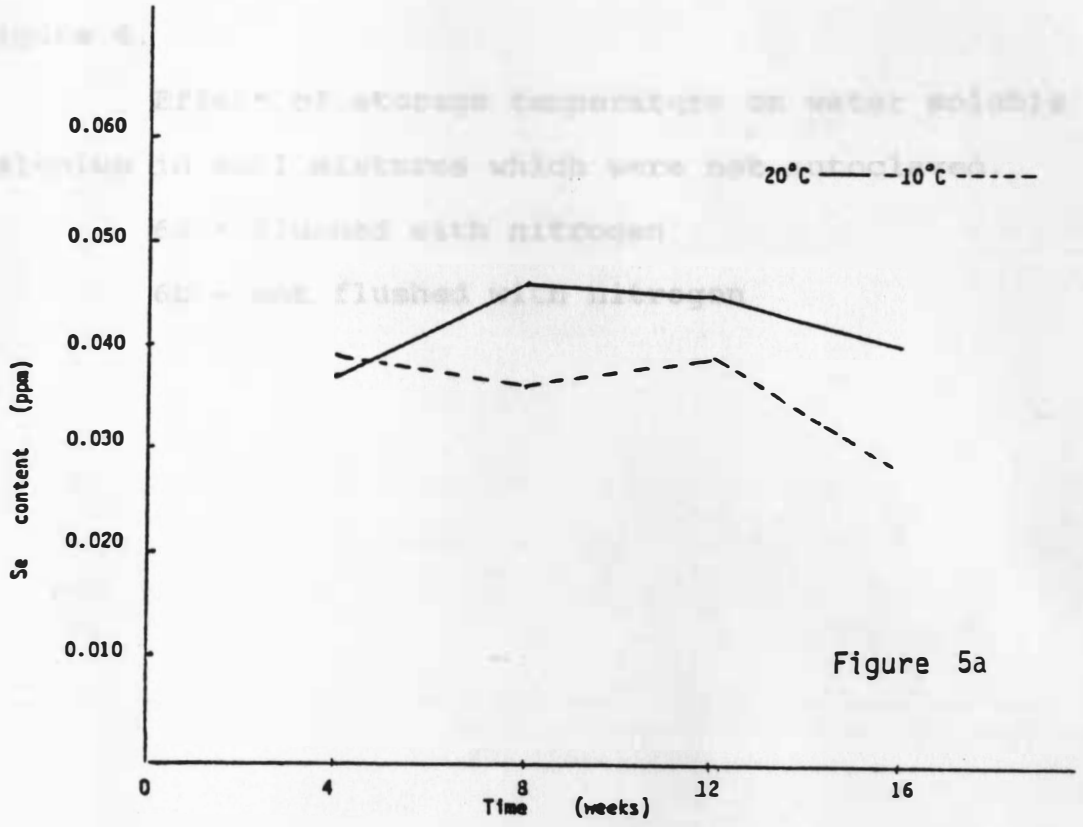


Figure 5a

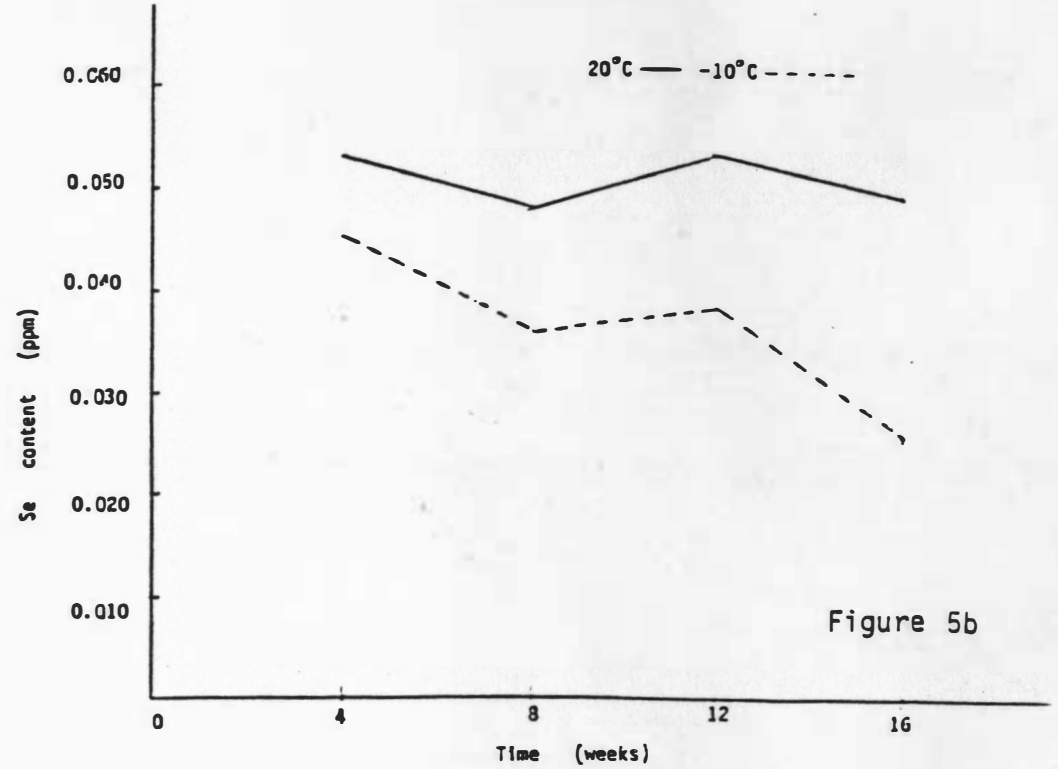


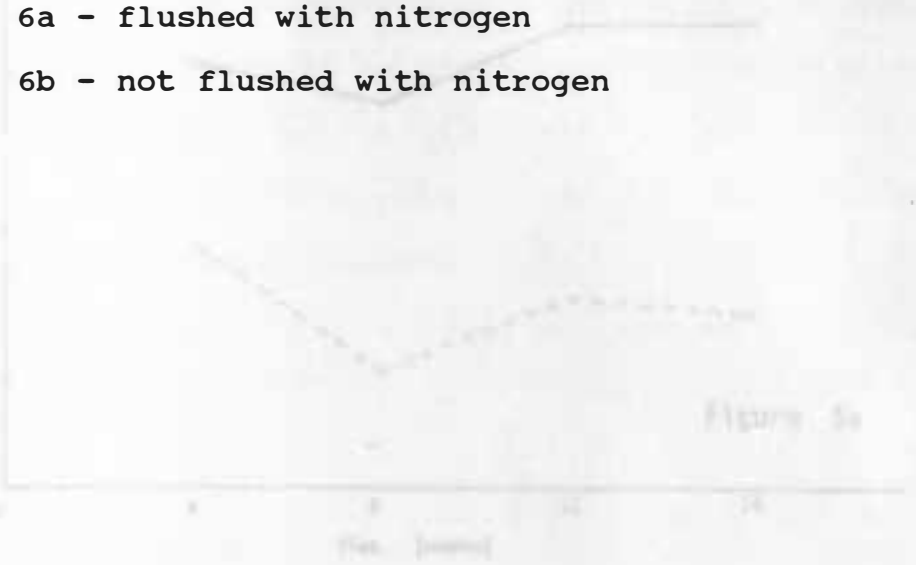
Figure 5b

Figure 6.

Effect of storage temperature on water soluble selenium in soil mixtures which were not autoclaved.

6a - flushed with nitrogen

6b - not flushed with nitrogen



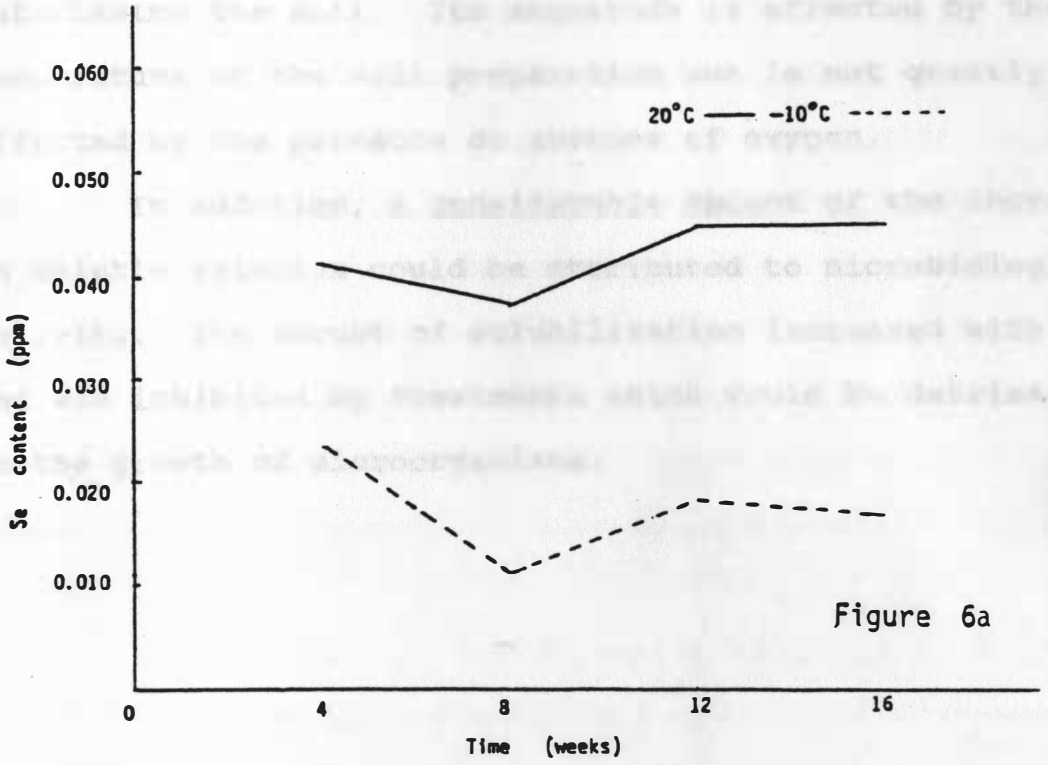


Figure 6a

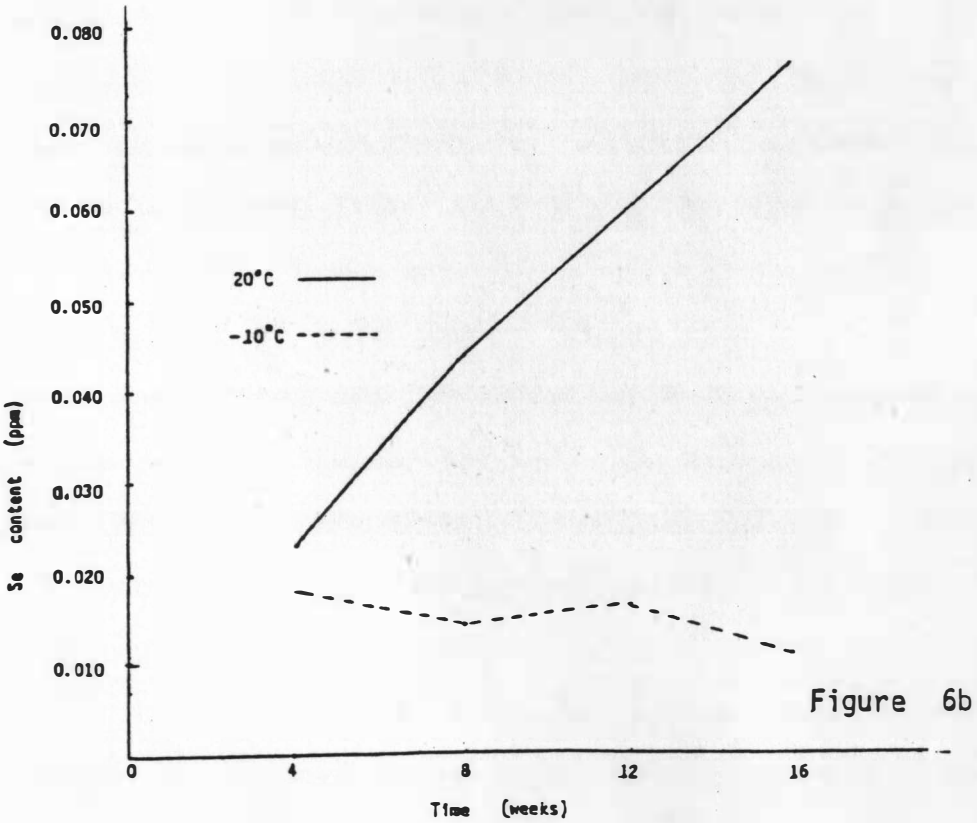


Figure 6b

autoclaving the soil. Its magnitude is affected by the temperature of the soil preparation but is not greatly affected by the presence or absence of oxygen.

In addition, a considerable amount of the increase in soluble selenium could be attributed to microbiological activity. The amount of solubilization increased with time and was inhibited by treatments which would be detrimental to the growth of microorganisms.

CONCLUSIONS

Selenium is thought to be an essential element for humans and animals which can be either beneficial or harmful depending upon the dosage used. Selenium-responsive diseases often occur in selenium deficient areas which produce crop plants containing selenium levels inadequate for the dietary needs of farm animals. Similarly high selenium areas will produce toxic vegetation which will cause selenium toxicosis in farm animals restricted to those feeds.

The practice of adding sodium selenite to the soil or to feedstuffs is of limited use due to the high cost of selenium salts. For economic reasons, fly ash, a waste product of coal-fired electricity generating plants has been studied as a selenium source. Appreciable amounts of selenium have been found in fly ash, mainly as elemental selenium. There has been a question as to the rate of solubilization of this form of selenium.

This study has investigated whether elemental selenium can be solubilized and then absorbed in very small amounts by non-accumulator plants (wheat).

Based on the data presented here, several conclusions can be deduced.

1. Elemental selenium added to soil may be converted to soluble forms more rapidly than was thought before.

2. In at least the soil used in the study, conversion of elemental selenium to available form can be rapid and extensive enough to produce selenium content in the experimental plants which would be considered toxic to animals (up to 35 ppm).

3. Different parts of the plant can accumulate different levels of available selenium. The grain accumulates more than the straw.

4. The drying of fresh vegetation at 70°C does not cause the loss of selenium compounds in wheat plants.

5. The solubilization of elemental selenium is contributed mainly by microorganisms and chemical reaction with the soil. Atmospheric oxidation makes little contribution except to enhance aerobic microbiological activity.

6. The use of fly ash as a soil amendment in agriculture to produce desired selenium levels in the utilizable plants is supported by the work and should be further investigated.

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